ONLINE SUPPLEMENTS

Mechanism of protein decarbonylation

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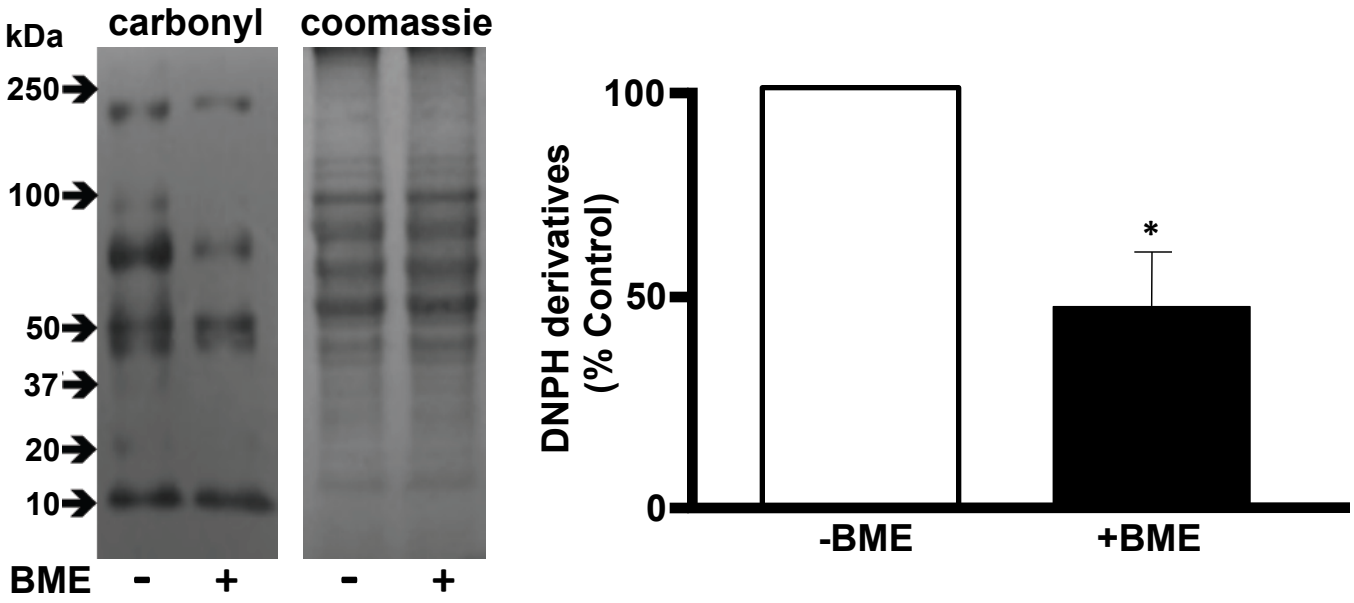
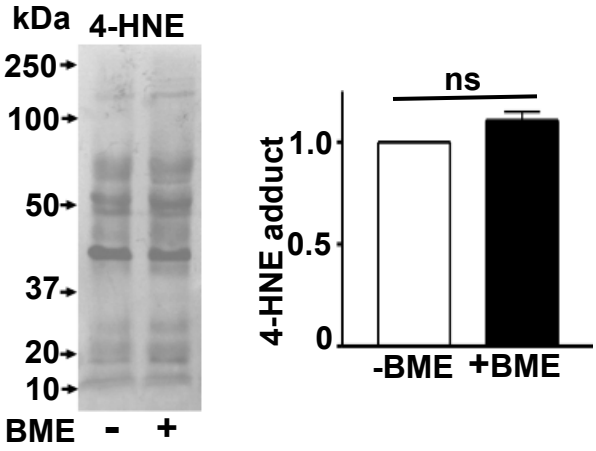
Supplemental Figure S1



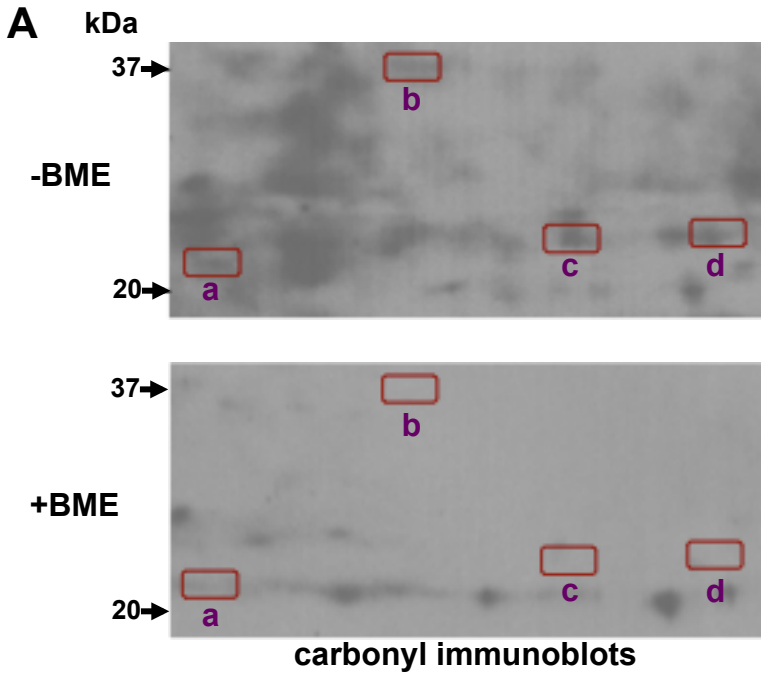
Supplemental Figure S1: Effects of BME on protein carbonylation in mouse heart tissue homogenates. Mouse heart homogenates were treated with or without 2% (w/v) BME, then derivatized with DNPH to monitor carbonylated protein content by immunoblotting. The bar graph represents means ± SEM (n = 3) of percent of carbonyl content relative to untreated control without BME treatment. The symbol * denotes that the value is significantly different from the control at $P < 0.05$. Coomassie stained proteins are also shown to demonstrate that BME did not influence total protein levels.

Supplemental Figure S2



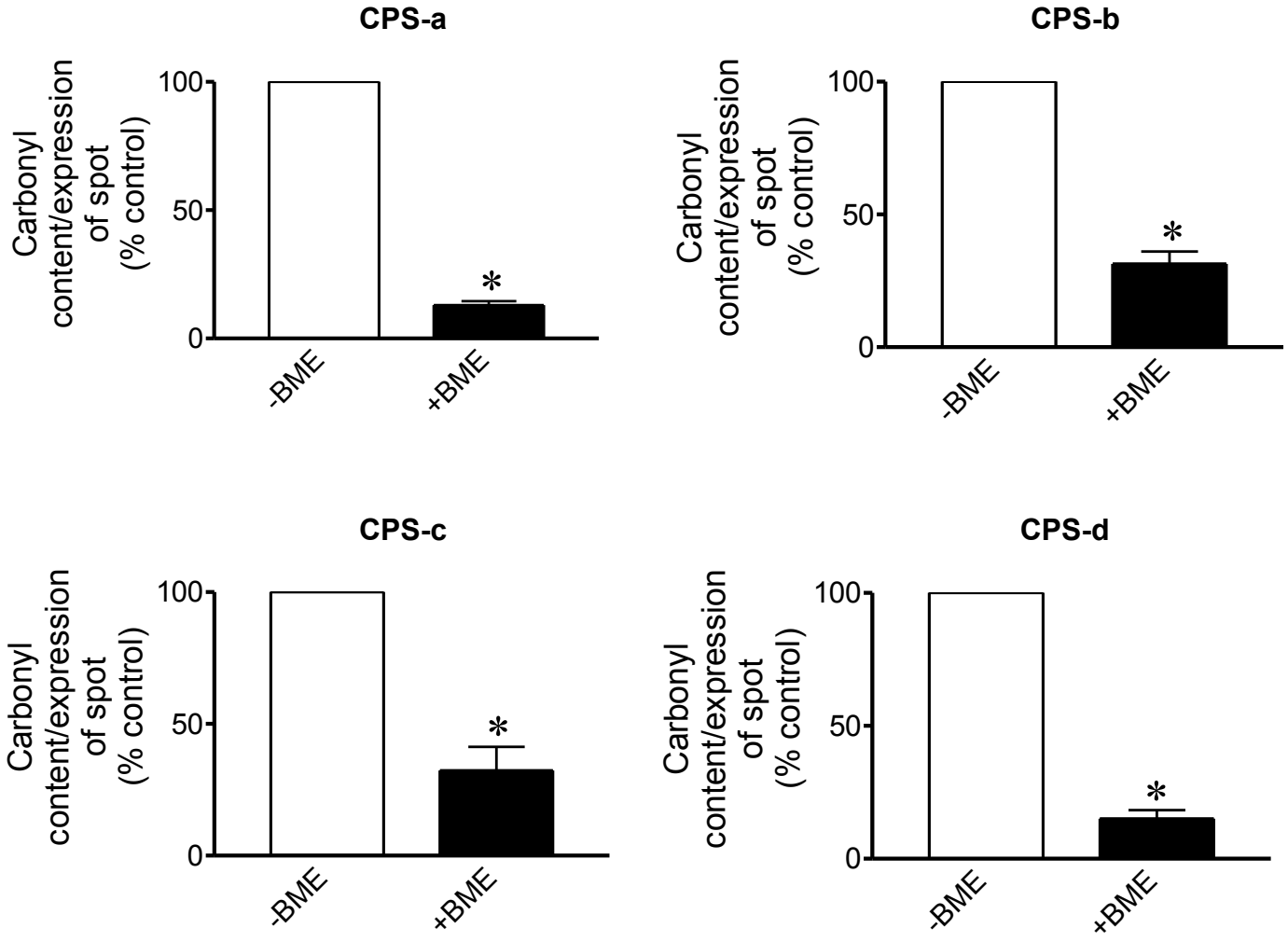
Supplemental Figure S2: Effects of BME on 4-HNE protein adducts. Rat heart homogenates were treated with or without 2% (w/v) BME, then subjected to immunoblotting to detect 4-HNE protein adducts (n = 9). The symbol ns denotes that the two values are not significantly different.

Supplemental Figure S3



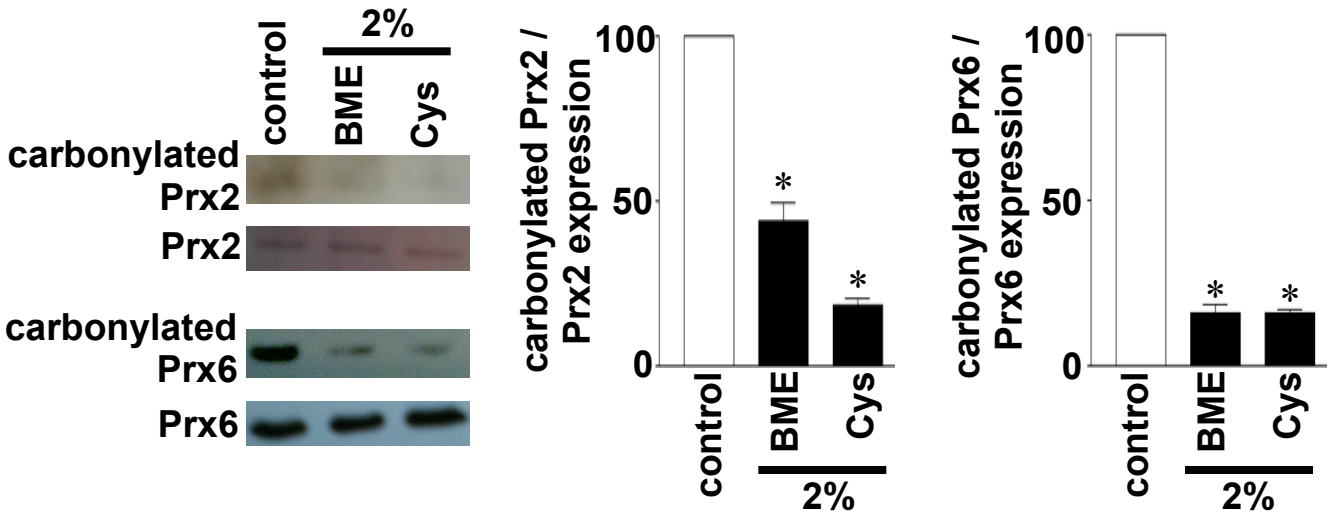
Supplemental Figure S3: Identification of proteins that undergo thiol-mediated reduction of protein carbonylation in heart tissue homogenates. Rat heart homogenates were treated with or without 2% (w/v) BME, then derivatized with DNPH to monitor carbonylated protein content using 2-D gel electrophoresis and immunoblotting. (A) Carbonyl immunoblots. (B) Coomassie Blue stained proteins. Carbonylated protein spots (CPS) that were analyzed by mass spectrometry are indicated in the red boxes.

Supplemental Figure S4



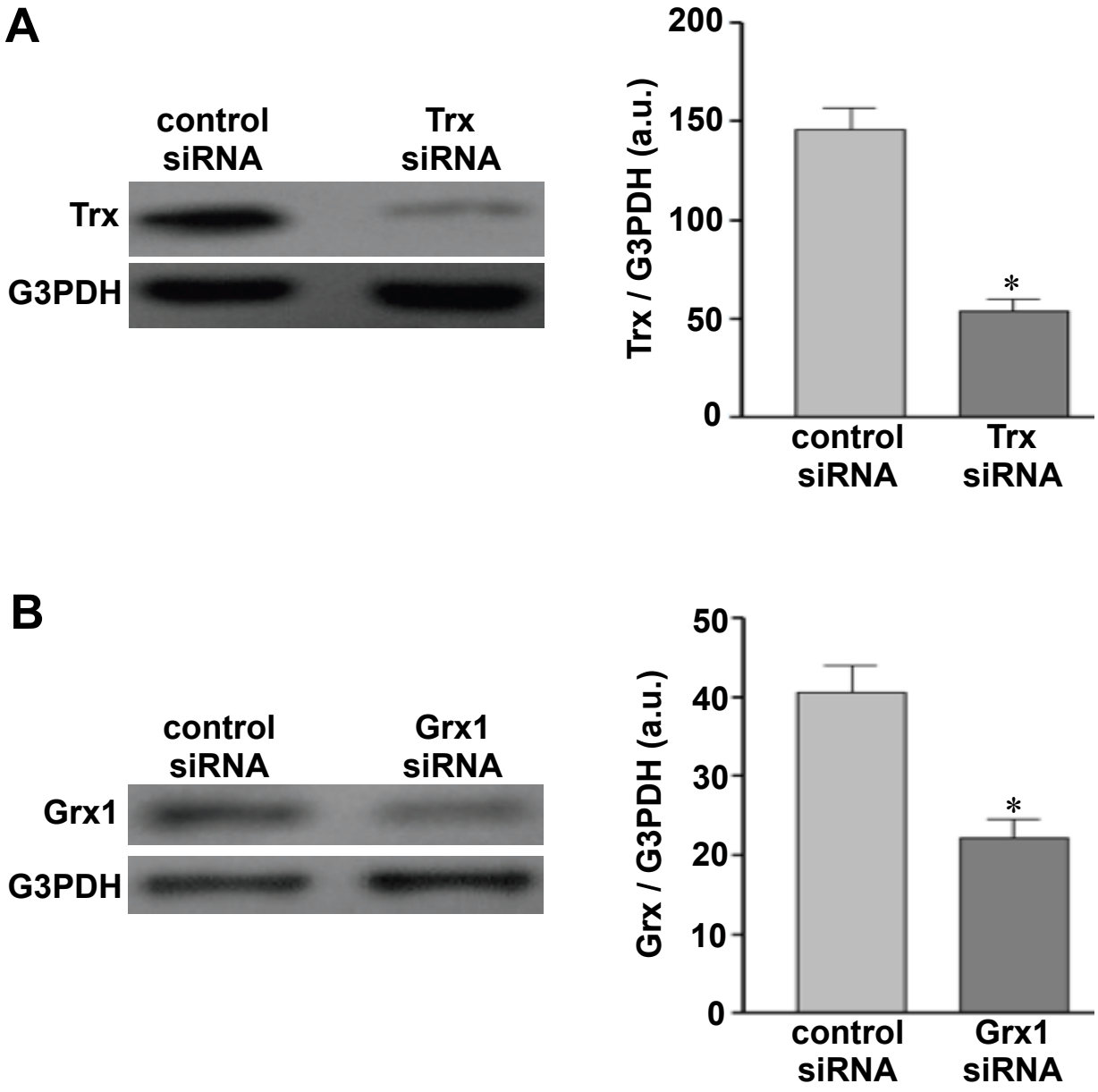
Supplemental Figure S4: Identifications of proteins that undergo thiol-mediated reduction of protein carbonylation in heart tissue homogenates. Rat heart homogenates were treated with or without 2% (w/v) BME, then derivatized with DNPH to monitor carbonylated protein content using 2-D gel electrophoresis and immunoblotting. Spots which exhibited significantly decreased carbonyl content in response to BME treatment were labeled as CPS in Supplemental Figure S3. Bar graphs represent means ± SEM (n = 3) of percent of carbonyl content/total protein relative to untreated control without BME treatment. The symbol * denotes that the value is significantly different from the control at $P < 0.05$.

Supplemental Figure S5



Supplemental Figure S5: Prx-2 and -6 undergo thiol-mediated reduction of protein carbonylation in heart tissue homogenates. Rat heart homogenates were treated with or without 2% BME or Cys, then derivatized with DNPH. Samples were immunoprecipitated with DNP antibody and immunoblotted Prx antibodies. Bar graphs represent means ± SEM (n = 3) of percent of carbonylated Prx/total Prx expression relative to untreated control without thiol treatment. The symbol * denotes that the value is significantly different from the control at $P < 0.05$.

Supplemental Figure S6



Supplemental Figure S6: siRNA knockdown of Trx and Grx1. Cultured human pulmonary artery SMCs were transfected with (A) Trx siRNA or (B) Grx1 siRNA for 2 days. Cell lysates were prepared and Trx and Grx1 protein levels were monitored by immunoblotting. G3PDH levels were also monitored as control. Bar graphs represent means ± SEM of the ratio of Trx or Grx1 to G3PDH expressed in arbitrary unit (a.u.). The symbol * denotes that values are significantly different from control siRNA values at $P < 0.05$.